In the cross $(B_1-;B+M+)(T-L-)V_1{}^s$ X $(B_1+;B-M-)(T+L+)V_1{}^r$, $V_1{}^r$ is more frequent both in B_1- and in prototrophs. It is linked therefore to (T,L). However, it is also linked to Lac, in the other linkage group. There is, therefore, but a single linkage group, of which the only suitable arrangement is: $B_1..(B,M)..Lac...V_1..(T,L)..$, the order of the factors within the parentheses being indeterminate from the present data.

Crossover theory

In an attempt to compute map distances from the available data, a crossover theory must be used in which, unfortunately, no correction for chiasma interference can be made. Such interference conceivably may result in large discrepancies between true and estimated values of map distance, particularly in the region (B,M) to (T,L).

As can be seen from the map, Table 6 , an interchange between B_1 and (B,M) results in $B_1+B+M+...$; lack of interchange in $B_1-B+M+...$ The ratio between these two types is 8:79. indicating a proportion of interchange to total of 8/79+8 or 9.2%. With so small a distance, correction for double or multiple crossovers would be negligible compared to the experimental error. With the formula developed below: tanh x = interchange: no interchange, a value of 10.1% would have been obtained.

The estimation of the distance (B,M) - (T,L) requires a detailed consideration of multiple crossing over. Absolute values for the distances $(B.M)$ - Lac; Lac - V_1 ; $V_1{}^-(TL)$, or a, b, c, respectively, are not available but only their relative proportions as given by the ratios of the single-crossover types in Table 6 . The values of a,b,c can however be estimated from their ratios, r_a , r_b , r_c and the proportion of the "triple crossover" class of table 6 , r_d, since the frequency of multiple crossing over will depend on the absolute map distances.

A recovered prototroph chromatid will fall into the classes a...d according to the distribution of crossover "breaks" in its various segments. Since interchange between B,M and T,L is required to produce a prototroph, only those chromatids with an odd number of breaks in the region a+b+c will be recovered.

The map distance, x, may be defined as (100 X) the mean number of crossover "breaks" in a segment. In the absence of interference, there should be a Poisson distribution of chromatids with varying numbers of breaks, the frequencies of 0,1,2,n, breaks being given by successive terms of the expression: e^{-x} (1, x, $x^2/2!$, $x^n/n!$). In this case, only chromatids representing the odd terms of this expansion can be considered, their sum being e^{-x} (sinh x) which is equivalent to $\dfrac{1 - e^{-2x}}{2}$. The sum of the even terms is e^{-x} (cosh x) whence the expression tanh x for the ratio of interchanges to non-interchanges. It can be shown from the addition formula for tanh x_1+x_2 that this formulation is equivalent to Haldane's addition formula $x_{12} = x_1 + x_2 - 2x_1x_2$.

The expression e^{-x}sinh x also applies to the chances of interchange in any segmental part of x, e.g. a, b, c. We have then, the four expressions following:

$$\frac{(e^{-a}\sinh a)(e^{-b}\sinh b)(e^{-c}\sinh c)}{e^{-x}\ \sinh x} = r_d = .020$$

$$\frac{(e^{-a}\sinh a)(e^{-b}\cosh b)(e^{-c}\cosh c)}{e^{-x}\ \sinh x} = r_a = .264$$

and so forth

Since $e^{-a} \cdot e^{-b} \cdot e^{-c} = e^{-x}$ appears in both numerator and denominator of each of these expressions, they can be cancelled out leaving only the hyperbolic terms:

$$\frac{\sinh a \cdot \sinh b \cdot \sinh c}{\sinh x} = .020$$

$$\frac{\sinh a \cdot \cosh b \cdot \cosh c}{\sinh x} = .264$$

$$\frac{\cosh a \cdot \sinh b \cdot \cosh c}{\sinh x} = .448$$

$$\frac{\cosh a \cdot \cosh b \cdot \sinh c}{\sinh x} = .268$$

$$\tanh a = \sqrt{\frac{a\,\delta}{b\,r}} \quad \cdots$$

While the solution of this system of equations would provide a theoretically exact solution for a, b, c and x, we are here concerned primarily with the estimation of x, and this can be more readily obtained with the help of certain approximations. In particular, **a** may be taken as $s_a x$, where s_a is $r_a/r_a + r_b + r_c$, i.e., the fraction of the "single" crossover types represented by a. This is not exact insofar as the proportions of the types a, b, c will not be directly related to the distances a, b, c in all cases, the contribution of the triple-crossover, single-interchange, types being as the 3rd powers of the distances, etc., rather than the first power.

The result of this approximation is the equation in one variable:

$$\frac{\sinh s_a x \cdot \sinh s_b x \cdot \sinh s_c x}{\sinh x} = .020 \text{ , which can be solved}$$

by successive approximations. The solution is x = .80, or 80 units (morgans). a, b, c are then 21+, 36+, and 22 units respectively.

As a check on the approximation used, the result of substituting a = b = c = x/3 may be considered. This leads to the equation:

$$\frac{\sinh^3 x/3}{\sinh x} = .020, \qquad x = .75, \text{ which considering}$$

the crudity of the approximation is in good agreement.

On the other hand the application of the uncorrected formula $\frac{(x/3)^3}{x} = .020$ gives the result, still lower, x = .73. This corresponds to counting single-crossovers only in the a, b, c types, and taking the three regions as equal in length.

The values already cited: 21+, 36+, and 22, will be taken as representing the best available estimate of the three regions.

Interference, by shifting the distribution of crossover types towards the lower values, would be expected to diminish r_d and therefore lead to a low estimate of x. However, interference would also tend to cause a spacing of crossovers, increasing the likelihood that where there are, for example, three crossovers, one shall be found in each segment and lead to a "d" type. With a random distribution of crossovers, there is only about 1 chance in 5 that 3 crossovers will be distributed 1:1:1; by interference this figure could conceivably be increased to 1/1, which would compensate for a fivefold bias against triple-crossovers compared to single-crossovers for a given value of x. Interference has not yet been sufficiently analysed in other organisms to permit of any more direct evaluation of the extent to which these effects will cancel each other. It would clearly be desirable to find other means of estimating these distances, perhaps by the use of biochemical markers located in the left hand region of the map. A comparison of the results might give direct information concerning the possible role of interference.

Linearity

In constructing a map, and calculating distances, it has been taken for granted that there is in E. coli a system of linear linkage, such as has been demonstrated quite conclusively in Drosophila, and inferred in all higher organisms. What direct evidence may one bring to bear on this question?

The method which one is forced to employ in hybridizing this bacterium introduces certain complications. The classical proof of linearity is based on the additive character of distances, expressed in morgans, between loci occurring within the same linkage group. The determination of map distances is based upon a comparison between

parental and new combinations of linked genes, as determined in the progeny of zygotes selected at random. In $\underline{E}$. $\underline{coli}$, on the other hand, one is limited to the recovery of that recombination class in which there has necessarily been an interchange between certain biochemical loci, in the cases here discussed, between (B,M) and (T,L).

The data analysed above, concerning the segregations of Lac and V_1 cannot be used for a demonstration of linear order without an error of circular reasoning. This is shown by the indeterminacy of interference which affects very vitally the linear additive properties of adjoining crossover segments.

The bearing of the reversed crosses tabulated in Tables 3 and 4 has already been mentioned. They illustrate the combinatorial character of the segregation mechanism but do not specify it more closely. For example, one might postulate that genes of bacteria are embedded in an n-dimensional matrix, which is ordinarily conserved, but which occasionally permits of a gene-for-gene interchange. This is equivalent to the "Konversion" theory once proposed by Winkler (1932) as an alternative to crossing-over theory in higher organisms, and which has been revived most recently, in modified form, by Lindegren (1947). The Konversion theory is made untenable by evidence for the interaction of different interchanges, for example, between Lac and V as already cited. The Konversion theory can be made to fit such results only by making it experimentally indistinguishable from the classical crossover theory.

The interactions between interchanges also exclude similar matricial theories where the units are perhaps not single genes, but blocks of them- for example a multilinear radial arrangement. At least for the genes involved in such interactions, one is forced to conclude that they are in a continuous segment. Other genes, as yet unstudied, of course might be shown to be placed on branches or

other bizarre modifications of the chromosome, but thus far no need for such exceptions has arisen.

Additional, perhaps more direct support for the linear order of genes is provided by data on the segregation of V_6 summarized in Table 8. It will be noted that the segregations of Lac, V_1 and V_6 are congruous between the B_1- and B_1+ classes (the latter in the sense discussed on p. 22). In the totals of "prototrophs" isolated from $B-M-T+L+B_1+Lac+V_1^r V_6^s$ X $B+M+T-L-B_1-Lac-V_1^s V_6^r$, one finds Lac- 78%, V_6^r 82%, and V_1^s 36%, indicating that, as in previous experiments, Lac is linked to (B,M) and V_1 to (T,L). In addition, V_6 is linked to (B,M) somewhat more intensely perhaps than is Lac. Since linkage to B_1 is already eliminated, one would predict from these totals, on the hypothesis of linearity, that Lac and V_6 should be linked, with V_6 to the left of Lac.

The data of Table 8 confirm this prediction. The parental couplings of Lac and V_6 are -r and +s respectively. Of 137 Lac-, 134 were V_6^r; of 39 Lac+, 29 were V_6^s. The order V_6 , Lac, V_1 is also supported, since the four most frequent types are those corresponding to single-crossovers on this basis, while they would include multiple-crossover classes with any other order.

Since Lac and V_1 are segregating, the totals for the four combinations of these two factors can be compared with those of previous experiments. A 4x2 table comparison with the corresponding cross, Table 5, row 1, gives $\chi^2 =$ 19.6 for three degrees of freedom, While this would be an exceedingly poor agreement if a normal distribution obtained, an analysis of variance by means of the variance ratio shows that p = .05 that the discrepancy can be accounted for in terms of the variance of the replicated experiments.

The first 8 factors tested, B,M,T,L,B_1,Lac, V_1, and V_6 have been shown to belong to the same linkage group. It is, there-

fore, extremely likely that there is but one linkage group in E. coli, the chances that another of the same magnitude exists being 2^{-7}, or .008. There is no cytological evidence to suggest more than one chromosome in E. coli. No other genetically investigated organism has so few linkage-groups. Cytologically, the nearest analogue is perhaps the compound chromosome of Ascaris megalocephalus v. univalens, 2n = 2.

Attempts to Induce Aberrations

Using a chromosomal theory as a working hypothesis, it was hoped that some verfication could be found by the study of types in which the normal order of genes was disturbed. Since there is only one chromosome (from the genetic evidence), the only types of re-arrangements would be changes, leading to a series of inversion types. It was thought that such types might be detected by genetical pro-cedures, by virtue of their effect on crossing over. In particular, the occurrence of an inversion in the region B_1- (B,M) would be ex-pected to have the effect of eliminating the recombination classes involving interchanges in this region. In the cross $B-M-T+L+B_1+$ x $B+M+T-L-B_1-$ this would be equivalent to the suppression of prototroph recombinants; B_1- types, however, would be recoverable, and allow the investigation of the extent of the changes.

Preliminary attempts to find such aberration types have, to date, been unsuccessful. The procedure was as follows:

Following treatment with nitrogen mustard or 20,000 r of x-rays, cells of Y-40 and of Y-53 were incubated separately for 24 hours, to allow the separation of cells or nuclei that might have been associated at the time of treatment. The cultures were then streaked out on nutrient agar plates. Single colonies of Y-40 were picked and streaked across a nutrient agar plate. Streaks of similarly treated Y-53 colonies were made from the opposite direction,

so that in the center of the plate, cells of the two types were mixed, treated colony by treated colony. The occurrence of colonies which would not interact to produce prototrophs, as detected by plating into minimal medium, would be an indicator that the combination was heterogeneous for an aberration. Since in these experiments, both "parents" were exposed to treatment, each plating was equivalent to the testing of two chromosomes, for the occurrence of an aberration. No marked variation in the yield of prototrophs was noted in tests involving 121 mustard and 28- X-ray-treated chromosomes. This can scarcely be regarded as an adequate sample in view of the stringent selection imposed by the technique, which might be expected to eliminate any aberration types which are even slightly less vigorous than the normal. This consideration is especially relevant in view of the "hemizygous" condition of any aberrations in the probably haploid vegetative cells. These studies will be continued.

How Many Segregants per Zygote?

In the experiments detailed in this paper, recombinants were obtained from different cell types which were first exposed to each other in an agar medium. Therefore, each prototroph recombinant colony seen by the experimenter marks the site of formation of a zygote. The question may immediately be raised whether there are at that site other recomination classes which, by virtue of their biochemical deficiencies, may not proliferate within the prototroph colony on the minimal selective medium. This is equivalent to inquiring whether there is but a single viable product of meiosis (as in megasporogenesis in many higher plants) or more than one, as in ascomycetes. The solution to this problem would be of special interest in relation to the possible occurrence of four-strand crossing over. In addition, if an appreciable proportion of prototroph colonies consisted of two distinct segregation types,

it would be necessary to isolate these types for the collection
of segregation data.

There are at least three ways in which a zygote might yield
more than one haploid recombinant. Firstly, the zygote might be
capable of proliferation in the diplophase (or sporophyte), leading
to the occurrence of several diploid cells, each of which might under-
go meiosis independently, and by chance yield several segregation
types. Secondly, a single zygote might produce, after meiosis, in
addition to the prototroph, the complementary multiple mutant class.
Thirdly, in a system of four-strand crossing-over, there might be two
supplementary prototroph recombinants differing in the segregation
of factors such as Lac and V_1 for which the diploid was heterozygous.

Obviously, the proper investigation of these possibilities
requires that one stringently avoid contamination of one colony with
another. For this reason, the cell-suspensions used were diluted so
as to yield only about 5-10 recombination colonies per plate.

Crosses were made between Y-40 and Y-53 (B-M-T+L+B_1+Lac+V_1^r
x B+M+T-L-B_1-Lac-V_1^s) on B_1- containing minimal agar medium. As
already noted, about 90% of the colonies from such a cross are
B+M+T+L+B_1-. The theoretical complementary class would be B-M-T-L-B_1+.
Because of its nutritional deficiencies, it could not be expected
to proliferate on the minimal medium even had it been produced after
meiosis. The possibility remains, however, that a few cells of
this constitution might still be present among the 10^8 or so B_1-
cells of the predominant type in a colony. By plating such colonies
into medium lacking B_1 but containing biotin, methionine, threonine
and leucine, the B_1- cells would be suppressed, while the postulated
multiple mutant type could form colonies and be recovered.

The experiment just described was carried out, testing 52
colonies for their content of other cell types. In general, a

thiaminless colony could be shown to contain from 10-100 cells capable
of forming colonies on the B,M,T,L medium. However, in each case
investigated these have been shown to be indistinguishable from the
Y-40 parental B-M-type, and must be presumed to arise from a sur-
prisingly low degree of contamination of the colony with these cells
from the heavily seeded plate. A few colonies were found which could
be characterized as reversions from B_1- to B_1+. These experiments
are, then, inclusive with respect to the occurrence of complimentary
geneotypes in the same colony. With appropriate stocks, not as yet
available, it should eventually be possible to manipulate the situation
so that the complementary type could be recovered selectively, ex-
cluding both parents and the dominant recombination class.

A search for supplementary types was conducted with the
same crosses, except that colonies appearing on B_1- agar were streaked
out directly on EMB-lactose agar to determine whether any of them
were heterogeneous for this factor. In some cases, a number of
isolated colonies from each EMB-test plate were then also tested for
homogeneity with respect to T1-resistance. About 90 colonies were so
tested; only 1 colony was found containing both Lac+ and Lac- cells.
It is impossible to be certain that, with this low frequency, the
single colony which was picked actually was derived from two distinct
zygotes. These experiments cannot be considered as bearing critically
on the question of the occurrence of two- or four-strand crossing
over because of the absence of information concerning the viability
of more than one meiotic product.

Diploidy.

The segregation of characters observed between prototroph
recombinants strongly suggests a haploid condition of E. coli, with re-
duction immediately following the zygotic fusion. If this condition
could be modified so as to yield stable diploid variants, which,

using with haploid cells, might yield zygotes showing tri-somic

egregation, many questions concerning centromere relations, and the

umber of strands as meiosis, as well as the dominance or recessive-

ess of particular characters, could be studied. Because of the

ifficulties of cytological examination, a genetic test was devised

hich, it was hoped, would detect stable diploid variants.

In 1913, Penfold, described a peculiar response of E. coli

o sodium chloroacetate. Wild type strains appeared to be inhibited

y this agent, but gave rise to resistant mutants, which appear as papill-

ae or button-like projections from the inhibited growth on nutrient

agar containing 0.1% sodium chloroacetate. The resistant mutants were

peculiar. insofar as they lacked the capacity to form gas from glucose,

although abundant acid was formed.

These findings were confirmed with E. coli, K-12. In addition,

it was found that while the resistant mutant, Cla^r, could form gas

from formate, it could not from pyruvate. The presently accepted

scheme for the formation of gas from carbohydrates by E. coli involves

the splitting of pyruvate to acetate, or similar C_2 fraction, and

formate. The formate is then decomposed to $CO_2 + H_2$ by the "formic

hydrogenlyase" complex. Since the capacity to form gas from formate

is intact, while that from pyruvate is impaired, it may be assumed

that there is a correlation between Cla^r and the enzymatic splitting

of pyruvate. While other interpretations are perhaps not ruled out,

this was adopted as the most likely explanation, and is the basis

for what follows.

On a priori grounds, and from the work of Beadle and Coon-

radt on Neurospora heterocaryons. it is likely that the ability

to perform a reaction will generally be dominant to the inability.

Cla^s therefore should be dominant in a diploid heterozygote to Cla^r

with respect to enzymatic function. If the correlation between

enzymatic function and resistance is maintained, Cla^S will also be dominant with respect to resistance, i.e., the combination Cla^S/Cla^r will still be sensitive to chloroacetate. Starting from the diploidised wild type, Cla^S/Cla^S, it will require two mutations to produce the resistant type Cla^r/Cla^r. On this basis, a diploidised E. coli should yield mutations to the phenotype of resistance to chloroacetate only with extreme infrequency compared to the normal haploid form. Since one need merely streak out cells of a suspected diploid type on chloroacetate agar, and record the development of papillae, this working hypothesis provides a possible tool for the detection of diploids. Unfortunately, none have yet been found among some dozens of tests of camphor- or acenaphthene- treated material. This matter was discussed primarily to illustrate a possibly very fruitful line for further research, particularly from the point of view of the possibility of cytogenetical correlations.

Transformation.   Experiments designed to extract transforming factors from cells of E. coli were mentioned in an earlier section. The failure or such experiments is in line with the genetical properties of the recombination system, linkage, etc., but cannot be regarded as conclusive for the exclusion of diffusible transforming factors. The methods used may have been too delicate to extract appreciable quantities or too rough to preserve what was extracted. There is, however, a further type of genetic experiment which bears on the possibility of transformation via soluble substances.

A glance at Table 7 shows that some "multiply-transformed" classes are more frequent than those involving changes of but one or two loci of one of the parents. On the transformation hypothesis, this might be interpreted in terms of the non-uniform susceptibility of different cells to transformation, so that wherever it takes place at all, it is likely to affect several genes. Under these

conditions, one would anticipate that susceptible cells might be influenced simultaneously by a mixture of transforming factors. On the transformation hypothesis for the exchanges described in this paper, it would be equivalent to predicting tri-parental recombinations in mixtures of three genetic cell-types.

A sexual mechanism has rather different consequence. Among other sexual organisms, biparental inheritance is the rule, barring the dubiously relevant exception of certain multiporic embryo-sac types in the angiosperms. In mixtures of three genetic types, only those types of zygotes may be inferred which result from pairwise fusions of cells. Zygote formation is so infrequent that the coincidence of successive fusions or the segregants of a Type 1 ✕ Type 2 zygote with a Type 3 gamete has a negligible likelihood.

This critical point of difference was subjected to experimental test in the following way. The same biochemical parents were used as before, namely B-M-, and T-L-B_1-. Lac and V_1 alleles were distributed among these parents in various combinations. For example, the types B-M-Lac-V_1^r ; T-L-B_1-Lac-V_1^s; and T-L-B_1-Lac+V_1^r were used. Suspensions of these types were prepared and all three mixed together in a manner analogous to that already described for normal, pairwise crosses. From such combinations, prototrophs or B_1-, other factors prototrophic, could be produced only by recombination between the B-M-parent and one or the other of the T-L-B_1- parents. By biparental inheritance, in this case, only three of the possible combinations of Lac and V could appear among the prototrophs: Lac-V_1^r; Lac-V_1^s; Lac+V_1^r. If a ménage à trois were permissible, however, the fourth type Lac+V_1^s should be found also.

By using different combinations of alleles, the experiment may be varied so that a different class becomes the exceptional in each case. In Table 9, the results of four experiments, so constructed

that a different class should be lacking on the basis of biparental
inheritance in each experiment, are set down. It will be seen that
no exceptional types appeared in a total of 628 tests. It may be con-
cluded that genetic factors from different cells are not freely
miscible, as would be demanded by the simplest versions of transfor-
mations. On the other hand, gene recombination is restricted in
any instance to exchanges of genetic material between two cell types.

The results of this experiment are also a check on spontaneous
mutation as the source of what have been claimed to be recombinations.
On the spontaneous mutation hypothesis there should be no discrimina-
tion against the exceptional types which were not found in these ex-
periments.

The genetics of bacterial transformations is still in an
exceedingly primitive state, and there is no information concerning the
occurrence of interactions in bona fide transforming systems, If
transformation is to account for the results of the present experiments
it will have to fulfill the following conditions: a) non-independence
of factors, simulating a linkage group; b) potential capacity of
carrying all the genetic factors of the donor in a single parcel;
c) immiscibility of parcels derived from different cells. From a
genetic point of view, such a transforming factor would be indistin-
guishable from a gamete, and its definition would be based on chemical
properties only. It will be recalled, however, that Muller (1947)
has interpreted the pneumococcus transformation in similar terms:
"still viable bacterial chromosomes or parts of chromosomes floating
free in the medium....these have penetrated the capsuleless bacteria
and, in part at least, taken root there, perhaps after having under-
gone a kind of crossing-over with the chromosome of the host."

Further genetic work on transforming systems will be required
to substantiate this interpretation, particularly in view of the

relatively low molecular weight, 500,000, which has been ascribed to
the pneumococcus factors. (Avery et al. 1944).

Other E. coli strains

        In an attempt to find how generally the ability the recombine
is distributed among bacteria, studies were made on two other strains
of E. coli. These were designated B/r and L-15. B/r, obtained through
the courtesy of Dr. E. Witkin, is a radiation-resistant mutant of
strain B. Both B and B/r have been used extensively in studies on
mutation from phage-sensitivity to phage-resistance. L-15 is a strain
used by Roepke, Libby and Jones (1944) for the production of biochemical
mutations. A variety of biochemical mutants of L-15 were obtained through
the courtesy of Dr. Roepke. Double mutants of B/r, arginine-methio-
nineless and histidine-p-aminobenzoieless, were obtained from ultra-
violet treated material by previously described techniques for the
isolation of mutants, Lederberg and Tatum, (1946a) All combinations of
the T-L-B_1- mutant, Y53, of K-12, and of the mutants of B/r and of L-15
were made and plated into minimal medium as already described. In no
case was there any suggestion of the formation of prototrophs within
L-15 or B/r mutants, between them, or with K-12. It is recognized that
the conditions for recombination in those strains may differ from K-12,
or that there may be genetic conditions of mating type. It was esti-
mated that recombination would have been detected had it occurred with
a frequency of not less than 10^{-3} of that found in K-12 mutants.

        Serious cytological studies seeking to identify the zygote
in K-12 have not been attempted in view of the futility of attempts
to characterize and verify so rare an occurrence. The burden of this
investigation has been the verification of the recombination of genes
in a bacterium, and the elucidation of some of its genetic properties.
The way is open for considerable further work, using recombination as
a tool of genetic analysis, and to the more detailed picturization of

process in space and time.

Discussion

Although a transformational interpretation of these experiments
has not been excluded beyond any shadow of doubt, it makes little
difference for most purposes whether one adheres to invisible zygotes
as against unextractable transforming factors. The techniques de-
scribed should be useful in either case toward the solution of genetic
problems in bacteria. Many of these are discussed in the Cold Spring
Harbour Symposium for Quantitative Biology, Volume 11, 1946, which
deals with the genetics of microorganisms. These problems include
the genetic nature of phenotypically complex mutations, the verification
of reverse mutation as the basis of genotypic reversion, the site of
interaction of certain mutations involving glycolytic enzymes, the
genetic basis of antigenic variations, the verification of the "one-to-
one" theory of the relationship between genes and enzymes in bacteria,
and in general, any instance where it is required to test the allelism
of two or more genetic variations.

Genetic recombination has, of course, a far broader meaning in
biology than as a laboratory tool. The recombination of mutations is
a source of variation that may be of crucial importance in the evolu-
tion of new "adaptive peaks". While this statement, in its general
terms is indubitable (see Dobzhansky 1941) recombination can only
effect the reshuffling of preexistent mutations. Concerning the natural
historical significance of the latter for bacteria, we are in a state
of woeful ignorance, so that we are hardly in a position to discuss
the significance of bacterial recombination in concrete terms. To this
must be added the caution that genetic combination was found, luckily,
in one E. coli strain, although not in two others which were studied.

It may be wondered at that the apparent recombination rate is so low. It will be recalled that about 10^{-6} of the cells insoculated in the cross B-M-T+L+B$_1$+ X B+M+T-L-B$_1$- showed interchange in the region (B,M) - (T,L). Since the estimated map distance is 80 units this is also the correct order of magnitude of the fusion process. However, this is possibly not to be ascribed to any sexual imperfections of the colon bacterium, but to the method of enumeration. It seems likely that an analogous comparison of the number of somatic and generative cells in a higher plant, or the ratio of perithecia to total nuclei in a fruiting culture of Neurospora would not give very different ratios. It is also possible that the optimal conditions for zygote formation and germination have not yet been achieved and that by special procedures the rate of fusion may be accelerated to the level where there might be some hope of trapping it in the field of the microscope.

Since the mutants used in the recombination experiments were derived from the same wild-type strain, there can be no question of genotypic mating type determination. The failure of two E. coli strains to exhibit recombination might conceivably be ascribed to genetic heterothallism, such as has caused many fungi to be classified as "imperfect". The chances of finding the appropriate mates are of course very slim, but should not be entirely overlooked.